(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: 0 293 158 A2

(12) **EUROPEAN PATENT APPLICATION**

(21) Application number: 88304670.8

(51) Int. Cl.4: C07K 7/10 , A61K 37/64 , G01N 33/68

(22) Date of filing: 23.05.88

Claims for the following Contracting State: ES.

(30) Priority: 26.05.87 US 54459

(43) Date of publication of application: 30.11.88 Bulletin 88/48

(84) Designated Contracting States: AT BE CH DE ES FR GB IT LI LU NL SE

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(54) Parathyroid hormone antagonists.

(57) The present invention relates to the use of peptide hormone analogues as inhibitors of their respective naturally occurring peptide hormone. The structure of the peptide hormone analogues is exemplified by Parathyroid hormone wherein Gly^{12} is substituted by an amino acid selected from the group consisting of L-Pro, D-Pro, L-Ala, D-Ala, Aib and NMeGly.

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PARATHYROID HORMONE ANTAGONISTS

BACKGROUND OF THE INVENTION

This invention relates to the use of peptide hormone analogues for inhibiting the naturally occurring hormone peptide in vivo and in vitro. These peptide hormone analogues when administered to a vertebrate, such as mammals, block the endrocrine activity of the peptide hormone or other analogous molecules. These peptide hormone analogues are also useful in vitro in combination with a bioassay for the naturally occurring hormone. The peptide hormone analogues are useful in treating various diseases caused by hormone excess and in treating hormone dependent tumors. One example of this invention relates to the synthesis of parathyroid hormone analogues useful for inhibiting the action of parathyroid hormone both in vivo and in vitro.

Analysis of the relation of structure to hormonal function has provided important insights into the mechanism of action of peptide hormones. Each type of peptide hormone has an affinity for specific receptors to which it binds. Upon binding, the peptide hormone acts either directly or causes a change in the intracellular concentration of a second messenger molecule such as cyclic AMP, cyclic GMP, or calcium ions. These second messenger molecules, in turn, cause changes in the metabolism or physiology of the cell. These changes in cell metabolism or physiology are directly or indirectly dependent upon the binding of the peptide hormone to its specific cell surface receptor. Therefore, if the cell surface receptor is blocked then the hormone effect is also blocked.

Peptide hormone analogues have long been known as a method through which the biochemistry of hormones can be studied and evaluated. Endocrinologists have long desired a method for producing a class of peptide hormone analogues which would allow the blocking of specific hormone receptors without activating a change in the second messenger molecules, thereby avoiding the hormone induced metabolic changes.

Rosenblatt et al., U.S. Patent 4,423,037 and the publications referred to therein describe the structure of certain peptide hormone analogues and their binding to cell receptors. In particular, these publications describe the properties of parathyroid hormone analogues and their physiological properties.

Scientific efforts over a period of many years have sought to understand the interaction between peptide hormones and the cell surface receptor specific for each peptide hormone. One of the peptide hormones, parathyroid hormone, has been studied by using analogues of parathyroid hormone (PTH). One objective of these studies has been to understand the binding of the peptide hormone to the cell surface receptor such that an analogue could be constructed which would bind with the same or greater affinity than the naturally occurring hormone. This analogue would enable the peptide hormone analogue of parathyroid hormone to be used to block the effect of the naturally occurring parathyroid hormone. One of the major problems encountered in this search for a clinically and pharmacologically effective parathyroid hormone analogue was the problem of agonist activity. Agonist activity is the property of the peptide hormone analogue to itself stimulate the change in second messengers which brings about the physiological change associated with the naturally occurring hormone. Therefore, the problem was to create hormone analogues which would bind with high affinity to the appropriate hormone cell surface receptor but not stimulate a change in the second messenger concentration, that is, not act as hormone itself. These analogues could then be used in treating hormone related diseases.

Secondary structure analyses of PTH have postulated a beta-turn in PTH at the region around position 12. Chou, P.Y. et al., Biophys, J. 26: 367-373 (1979). The present invention accomplished a series of substitions of amino acids in this region in order to stabilize the secondary structural conformation. It is, therefore, a primary object of the present invention to stabilize the conformation of PTH in order to enhance the biological activity of PTH analogues.

Another object of the present invention is to provide novel PTH analogues. Another object of the present invention is to provide a method of inhibiting the action of PTH through the administration of novel PTH analoges. Still another object of the invention is to provide PTH analoges wherein amino acid modifications result in binding to all the surface receptor without activating the second messenger molecule. The above and other objects are accomplished by the present invention in the manner more fully described below.

SUMMARY OF THE INVENTION

The present invention provides a peptide which comprises PTH(1-34)NH_2, [Tyr^{34}]PTH (1-34)NH_2, [$Nle^{8,18}$, Tyr^{34}]PTH(1-34)NH_2; PTH(3-34)NH_2, [Tyr^{34}]PTH(3-34)NH_2, [$Nle^{8,18}$, Tyr^{34}]PTH(3-34)NH_2; PTH(4-34)NH_2, [Tyr^{34}]PTH(4-34)NH_2, [$Nle^{8,18}$,Tyr^{34}] PTH(4-34)NH_2; PTH(5-34)NH_2, [Tyr^{34}]PTH (5-34)NH_2, [$Nle^{8,18}$, Tyr^{34}] PTH(5-34)NH_2; PTH(7-34)NH_2, [Tyr^{34}]PTH(7-34)NH_2, [$Nle^{8,18}$,Tyr^{34}]PTH(7-34)NH_2 wherein Gly^{12} is substituted by an amino acid selected from the group consisting of L-Pro, D-Pro, L-Ala, D-Ala, Aib (aminoisobutyric) and NMeGly. The PTH can be human parathyroid hormone (hPTH), bovine parathyroid hormone (bPTH) or rat parathyroid hormone (rPTH). Some representative examples of the peptide analogues of the present invention, are as follows:
[L-Ala^{12}]hPTH(7-34)NH_2, [L-Ala^{12},Tyr^{34}]hPTH (7-34)NH_2, [L-Ala^{12}, $Nle^{8,18}$, Tyr^{34}]hPTH (7-34)NH_2; [D-Ala^{12}]-hPTH(7-34)NH_2, [D-Ala^{12}, Tyr^{34}]hPTH(7-34)NH_2, [D-Ala^{12}, $Nle^{8,18}$, Tyr^{34}] hPTH(7-34)NH_2; [Aib^{12}]h PTH(7-34)NH_2, [Aib^{12},Tyr^{34}]hPTH(7-34)NH_2, [Aib^{12}, $Nle^{8,18}$, Tyr^{34}]hPTH(7-34)NH_2. These representative examples should not be construed as limiting the invention.

The present invention also provides a method of inhibiting the action of parathyroid hormone comprising the administration of therapeutically effective amount of a parathyroid hormone analogue described above. The present invention also provides a method of treating osteoporosis or hypercalcemia comprising the administration of a therapeutically effective amount of a parathyroid hormone analogue described above. A method of treating hyperparathyroidism comprising the administration of a therapeutically effective amount of the parathyroid hormone analogues of this invention is also provided. A method of treating hyperparathyroidism expressed as a hypercalcemic crisis, renal failure or hypertension is also provided. A method of treating the disease state produced by a tumor or other cell overproducing a peptide hormone-like molecule and method of treating immune diseases wherein the disease state comprises inflammation, an allergic response, or hyperactive lymphocytes is also provided by the novel peptide hormone analogues of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

Various other objects, features and attendent advantages of the present invention will be more fully appreciated as the same becomes better understood from the following detailed description.

Extensive structure and activity studies have now led to the design of peptide hormone analogues which have high binding affinity for their respective cell surface receptors while not stimulating the production of second messenger molecules. An example of such a peptide hormone analogue is [Aib^{12}, Tyr^{34}]hPTH(7-34)NH_2 amide which inhibits PTH in vivo but does not act as an agonist.

Agonist activity is dependent upon the presence of the N-terminal amino acid sequence. The removal two to six end terminal amino acids results in the loss of most if not all agonist activities. Therefore, the second messenger molecules are not affected by those analogues which have the altered amino terminus.

PTH analogues with two to six amino acids removed from the N-terminus produces an inhibitor which still binds with high affinity to the peptide hormone receptor without causing a change in cyclic AMP concentration.

The following is the 34-amino acid sequence of bovine parathyroid hormone (bPTH):
H2N-ALA-VAL-SER-GLU-ILE-GLN-PHE-MET-HIS-ASN-LEU-GLY-LYS-HIS-LEU(15)-SER-SER-MET-GLU-ARG-VAL-GLU-TRP-LEU-ARG-LYS-LYS-LEU-GLN-ASP(30)-VAL-HIS-ASN-PHE-COOH.

The following is the 34-amino acid sequence of human parathyroid hormone (hPTH):
H2N-SER-VAL-SER-GLU-ILE-GLN-LEU-MET-HIS-ASN(10)-LEU-GLY-LYS-HIS-LEU-ASN-SER-MET-GLU-ARG(20)-VAL-GLU-TRP-LEU-ARG-LYS-LYS-LEU-GLN-ASP(30)-VAL-HIS-ASN-PHE-COOH.

The following is the 34-amino acid sequence of rat parathyroid hormone (rPTH):
H2N-ALA-VAL-SER-GLU-ILE-GLN-LEU-MET-HIS-ASN(10)-LEU-GLY-LYS-HIS-LEU-ALA-SER-VAL-GLU-ARG(20)-MET-GLN-TRP-LEU-ARG-LYS-LYS-LEU-GLN-ASP(30)-VAL-HIS-ASN-PHE-COOH.

Fragments of peptide hormones containing the region specific for binding to the cell surface receptor can be used as inhibitors or blocking agents. For parathyroid hormone, the N-terminal 34 amino acids are sufficient to define binding specificity to the parathyroid hormone cell surface receptor. This receptor specificity is further defined by the following publication herein incorporated by reference: M. Rosenblatt, et al., Endocrinology, 107:2, 545-550, 1980 and S. R. Nussbaum, et al., Journal of Biological Chemistry, 255:10183, 1980.

The presence of D-amino acids in peptide hormone in place of L-amino acids results in a peptide resistant to catabolism. However, not all such substitutions result in an active peptide hormone. The

insertion of D-tyrosine at position 34 in PTH results in a significant increase in the biological activity of the hormone in addition to increasing stability of the peptide. The utilization of D-amino acids in peptide hormone synthesis is described in the following publications herein incorporated by reference: Coltrera, et al., Biochemistry, 19:4380-4385, 1980; Rosenblatt et al., Biochemistry, 20:7246-7250, 1981.

The balance of the description will be divided into two sections. Section I will describe the preparation and structure of inhibitors of peptide hormones, Section II will discuss the use of the peptide hormone inhibitors.

I. Preparation and Structure of Peptide Hormone Inhibitors

The technique of solid-phase peptide synthesis, developed by Merrifield ("Solid-Phase Peptide Synthesis", Advances in Enzymology, 32:221-296, 1969) has been successfully employed in the synthesis of peptide hormones including parathyroid hormone. This method is based on the strategy of having the carboxyl terminus of the peptide linked covalently to a solid support. The desired peptide sequence is prepared by stepwise coupling of single amino acids to a peptide chain growing from the carboxyl toward the amino terminus. Because each amino acid is coupled by nearly the same series of reactions, the need for elaborate strategies in the synthesis is minimized. Solubility is not a major issue during synthesis, because the peptide is linked to a solid support. This method is rapid and it can be utilized by a single worker. It is very convenient for the synthesis of multiple analogues with amino-terminal substitutions, because a single synthesis can be branched in multiple directions near the amino terminus, thereby creating many analogues varying only in the amino terminal region.

II. Use of Peptide Hormone Inhibitors

The method of inhibiting the action of peptide hormones comprises the administration of a therapeutically effective amount of any peptide hormone or analogue wherein the two N-terminal amino acids are removed and zero or more of the next four N-terminal amino acids are removed sequentially from the N-terminus. These hormone analogues retain specificity for the cell surface receptor without stimulating a physiological response. This method of use applies to the entire peptide hormone or its analogue, or to a fragment of the peptide hormone containing the receptor binding site.

The use of peptide hormone analogues is exemplified by parathyroid hormone analogues. The parathyroid hormone may be of bovine, human, rat, or any vertebrate origin. The analogue may contain all the amino acids except for the modified N-terminal region or it might comprise the N-terminal 7-34 amino acids. Individual amino acids can be substituted to improve stability as exemplified by tyrosine or norleucine in the present invention.

The peptide hormone analogues of this invention can be used in vitro to measure the concentration of naturally occurring peptide hormone. This bioassay procedure is illustrated by a bioassay for parathyroid hormone. The unknown concentration of parathyroid hormone in a solution can be determined by measuring the amount of parathyroid hormone analogue required to inhibit its binding to the parathyroid hormone cell surface receptor. The concentration of PTH analogue required to block the action of parathyroid hormone is a direct indicator of the parathyroid hormone concentration.

Parathyroid hormone analogues can be used to diagnose the etiology of or to treat osteoporosis or hypercalcemia through the administration of a therapeutically effective amount of the parathyroid hormone analogues of this invention. Similarly, hyperparathyroidism and other aspects of hyperparathyroidism, such as a hypercalcemic crisis, renal failure or hypertension can be treated through the administration of the parathyroid hormone analogues of this invention.

Tumors and other aberrant cell growth often produce hormone like substances causing a disease state. The use of peptide hormone analogues to block stimulation caused by such hormone like substances can result in the alleviation of the disease state. Therefore, the peptide hormone analogues of the present invention can be administered to treat diseases caused by aberrant production of hormone like substances.

Immune diseases such as inflammation, allergic responses and hyperactive lympocytes can be treated through the administration of peptide hormone analogues which block the action of peptide hormones, such as PTH analogues inhibiting the binding of PTH to cells of the immune system.

The peptide hormone analogues of this invention exhibit both oral and parenteral activity and can be formulated in dosage forms for oral, parenteral, rectal, intra-nasal, or topical administration. Solid dosage forms for oral administration include capsules, tablets, pills, powders and granules. In such solid dosage

forms, the active compound is admixed with at least one inert diluent such as sucrose, lactose or starch. Such dosage forms can also comprise, as is normal practice, additional substances other than inert diluent. In the case of capsules, tablets, and pills, the dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with an enteric coating.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsion, solutions, suspensions, syrups and elixers containing inert diluents commonly used in the pharmaceutical art. Besides inert diluents, such compositions can also include adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening. Preparations according to this invention for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions or emulsions. Examples of non-aqueous solvents or vehicles are propylene glycol, polyethylene glycol, vegetable oils such as olive oil and injectable organic esters such as ethyloleate.

Compositions for rectal administration are suppositories which may contain in addition to the active substance, expients such as cocoa butter or a suppository wax. The dosage of active ingredient in the compositions of this invention may be varied; however it is necessary the amount of the active ingredient shall be such that a suitable dosage form is obtained. The selected dosage form depends upon the desired therapeutic effect, on the route on the administration, and on the duration of the treatment.

Obviously, numerous modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described herein.

EXAMPLE 1

Synthesis and Purification of Peptide Hormone Analogues of PTH

Analogues of parathyroid hormone, were prepared by a modification of the solid-phase method of Merrifield. Syntheses were performed using an Applied Biosystems 430A Synthesizer. 4-Methyl benzhydrylamine hydrochloride resin (polystyrene-1% by divinylbenzene, USB) was employed as the solid support in order to effect the carboxyamide (CONH2) COOH-terminal modification.

The tertiary butyloxycarbonyl (Boc) group was used to protect the alpha -amino group of each amino acid during coupling. Side-function protection was afforded as follows: (a) the hydroxyl group of serine was protected as the O-benzyl ether; (b) the hydroxyl group of tryosine as the 0-2,6-dichlorobenzyl ether or p-bromobenzyloxycarbonyl ester; (c) the carboxyl group of glutamic and aspartic acid as the benzyl or cyclohexyl ester; and (d) the imidazole nitrogen of histidine by the benzyloxymethyl (BOM) and the guanidine function of arginine was protected by the p-toluene-sulfonyl group, and the indole imine by formyl groups. All amino acids were obtained from Applied Biosystems, Inc., Peninsula Laboratories, or Bachem Chemicals.

The peptide-resin synthesis were carried out using Applied Biosystems, Inc. specified protocols. Double couplings were carried out for the incorporation of each amino acid. Deprotection times with trifluoroacetic acid were extended 6 minutes over manufacturer protocols.

The peptide was cleaved from the copolymer resin with simultaneous removal of the side-chain protecting groups similar to the 2 step HF cleavage procedure described by Tam, J.A.C.S. 105: 6442-6455 (1983). In the first HF step the following ratios of reagents were used: 5% p-cresol, 5% p-thiocresol, 65% dimethyl sulfide and 25% HF. 10 ml of mixture per gram of peptide-resin was used for 2 hours at 0° C. In the second HF step the following ratio of reagents were used: 5% p-cresol, 5% p-thiocresol and 90% HF. The cleavage was carried out for 75 min. at 0° C. After removal of HF the peptide-resin mixture was washed with anhydrous ether to remove scavenger. The peptide was then extracted with 50% acetic acid and water. The washes were combined and chromatographed using Sephadex G-50F, eluting with 50% HOAc.

After lyophilization, the partially purified peptide was chromatographed by reverse phase HPLC (Vydac C_4 bonded silica, 15 u particle size, 300A pore size, using aqueous acetonitrile gradient containing 0.1%TFA).

EXAMPLE 2

PTH Binding Assay Results

PTH analogues were analysed in a new receptor assay which modified the assay reported in Rosenblatt et al., Endocrin. 107: 545-550 (1980). The binding assay used [$Nle^{8,18}$, ^{125}I-Tyr^{34}]bPTH (1-34)NH_2 which was purified by HPLC (Novapak C_{18}, 32-35% CH_3CN in 0.1% TFA) and was stored as aliquots in 25 mM TrisHCl/1%BSA at -70°C. Bovine renal cortical plasma membranes were incubated with radioligand (25,000 cpm) in a Tris-containing buffer (250 ul) for 30 min. at 21°C. Once equilibrium was reached, bound and free radioligand were separated by centrifugation. High specific binding (85%) to bovine renal cortical membranes was obtained consistently.

TABLE

Structure	Binding K_I (nM)
[Aib^{12},Tyr^{34}]bPTH(7-34)NH_2	51.0 ± 9.0
[D-Ala^{12},Tyr^{34}]hPTH(1-34)NH_2	0.9 ± 0.1
[L-Ala^{12},Tyr^{34}]hPTH(1-34)NH_2	1.0 ± 0.04
[Aib^{12},Tyr^{34}]hPTH(1-34)NH_2	0.8 ± 0.1
[Pro^{12},Tyr^{34}]hPTH(1-34)NH_2	587.0 ± 196.0

Claims

1. A peptide which comprises PTH(1-34)NH_2, [Tyr^{34}]PTH (1-34)NH_2, $Nle^{8,18}$,Tyr^{34}]PTH(1-34)NH_2; PTH(3-34)NH_2, [Tyr^{34}]PTH(3-34)NH_2, [$Nle^{8,18}$, Tyr^{34}]PTH (3-34)NH_2; PTH(4-34)NH_2, [Tyr^{34}]PTH(4-34)NH_2, [$Nle^{8,18}$,Tyr^{34}] PTH(4-34)NH_2; PTH(5-34)NH_2, [Tyr^{34}]PTH (5-34)NH_2, [$Nle^{8,18}$,Tyr^{34}] PTH(5-34)NH_2; PTH(7-34)NH_2, [Tyr^{34}]PTH (7-34)NH_2, [$Nle^{8,18}$,Tyr^{34}]PTH(7-34)NH_2 wherein Gly^{12} is substituted by an amino acid selected from the group consisting of L-Pro, D-Pro, L-Ala, D-Ala, Aib and NMeGly.

2. A peptide according to Claim 1 wherein said PTH is hPTH, bPTH or rPTH.

3. A peptide according to Claim 2 which is [L-Ala^{12}]hPTH(7-34)NH_2, [L-Ala^{12},Tyr^{34}]hPTH (7-34)NH_2, [L-Ala^{12},$Nle^{8,18}$, Tyr^{34}]hPTH (7-34)NH_2; [D-Ala^{12}]-hPTH(7-34)NH_2, [D-Ala^{12}, Tyr^{34}]hPTH(7-34)NH_2, [D-Ala^{12}, $Nle^{8,18}$, Tyr^{34}] hPTH(7-34)NH_2; [Aib^{12}]hPTH(7-34)-NH_2, [Aib^{12},Tyr^{34}]hPTH(7-34)NH_2, [Aib^{12}, $Nle^{8,18}$, Tyr^{34}]hPTH(7-34)NH_2.

4. A peptide according to Claim 2 which is [Aib^{12}, Tyr^{34}]bPTH(7-34)NH_2, [$NMeGly^{12}$, Tyr^{34}]bPTH(7-34)NH_2, [Aib^{12},Tyr^{34}]bPTH (7-34)NH_2, [D-Ala^{12}, Tyr^{34}]hPTH(1-34)NH_2, [L-Ala^{12},Tyr^{34}]hPTH(1-34)NH_2, [Aib^{12},Tyr^{34}] hPTH(1-34)NH_2, [Pro^{12},Tyr^{34}]hPTH(1-34)-NH_2.

5. The use of a peptide as claimed in Claim 1 for the preparation of a medicament useful for acting upon a PTH receptor.

6. The use as claimed in Claim 5, wherein said peptide is hPTH.

7. An in vitro bioassy of parathyroid hormone, wherein a measured amount of the analogue of Claim 1 inhibits binding a parathyroid hormone to a PTH receptor in vitro.

8. The use as claimed in Claim 5, wherein said medicament is for the treatment of hypercalcemia.

9. The use as claimed in Claim 5, wherein said medicament is for the diagnosing or treating of hyperparathyroidism.

10. The use as claimed in Claim 5, wherein said medicament is for the treatment of osteoporosis.

11. The use as claimed in Claim 5, wherein a tumor produces a parathyroid hormone-like substance.

12. The use as claimed in Claim 5, wherein said medicament is for the treatment of an immune disease.

13. The use as claimed in Claim 5, wherein said medicament is for the treatment of hypertension.

14. A pharmaceutical composition which comprises an effective amount of a peptide of Claim 1 and a pharmaceutically acceptable carrier.

Claims for the following Contracting State: ES

1. A process for preparing novel peptide PTH analogues that comprise PTH(1-34)NH_2, [Tyr^{34}]PTH (1-34)NH_2, [$Nle^{8,18}$,Tyr^{34}]PTH(1-34)NH_2; PTH(3-34)NH_2, [Tyr^{34}]PTH(3-34)NH_2, [$Nle^{8,18}$,Tyr^{34}]PTH (3-34)NH_2; PTH(4-34)NH_2, [Tyr^{34}]PTH(4-34)NH_2, [$Nle^{8,18}$,Tyr^{34}] PTH(4-34)NH_2; PTH(5-34)NH_2, [Tyr^{34}]PTH (5-34)NH_2, [$Nle^{8,18}$,Tyr^{34}] PTH(5-34)NH_2; PTH(7-34)NH_2, [Tyr^{34}]PTH (7-34)NH_2, [$Nle^{8,18}$, Tyr^{34}]PTH(7-34)NH_2 wherein Gly^{12} is substituted by an amino acid selected from the group consisting of L-Pro, D-Pro, L-Ala, D-Ala, Aib and NMeGly;
which process is carried out by a modification of the solid-phase method of Merrifield and is characterized by:
a) covalently linking the carboxyl terminus of the peptide to a solid support;
b) stepwise coupling of single amino acids desired, having protected both the α-amino group and the side-functions, to a peptide chain growing from the carboxyl towards the amino terminus; and
c) cleaving the desired peptide from the support with simultaneous removal of the side-chain protecting groups.

2. A process according to Claim 1 wherein said PTH is hPTH, bPTH or rPTH.

3. A process according to Claim 2 wherein the peptide obtained is selected from:
[L-Ala^{12}]hPTH(7-34)NH_2, [L-Ala^{12},Tyr^{34}]hPTH (7-34)NH_2, [L-Ala^{12},$Nle^{8,18}$,Tyr^{34}]hPTH (7-34)NH_2; [D-Ala^{12}]-hPTH(7-34)NH_2, [D-Ala^{12}, Tyr^{34}]hPTH(7-34)NH_2, [D-Ala^{12}, $Nle^{8,18}$, Tyr^{34}] hPTH(7-34)NH_2; [Aib^{12}]hPTH(7-34)-NH_2, [Aib^{12},Tyr^{34}]hPTH(7-34)NH_2, [Aib^{12},$Nle^{8,18}$, Tyr^{34}]hPTH(7-34)NH_2.

4. A process according to Claim 2 wherein the peptide obtained is selected from:
[Aib^{12},Tyr^{34}]bPTH(7-34)NH_2, [$NMeGly^{12}$, Tyr^{34}]bPTH(7-34)NH_2, [Aib^{12}, Tyr^{34}]bPTH (7-34)NH_2, [D-Ala^{12},Tyr^{34}]hPTH(1-34)NH_2, [L-Ala^{12},Tyr^{34}]hPTH(1-34)NH_2, [Aib^{12},Tyr^{34}] hPTH(1-34)NH_2, [Pro^{12},Tyr^{34}]hPTH-(1-34)NH_2.

5. A method of bioassay in vitro for determining the concentration of PTH in a solution, characterized by measuring the amount of a PTH analogue obtained according to claim 1 required to inhibit the binding of the PTH to the PTH receptor in vitro.

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: 0 341 962 A2

(12) EUROPEAN PATENT APPLICATION

(21) Application number: 89304646.6

(51) Int. Cl.[4]: C07K 7/10 , A61K 37/64

(22) Date of filing: 08.05.89

(30) Priority: 09.05.88 US 191513
09.05.88 US 191514

(43) Date of publication of application:
15.11.89 Bulletin 89/46

(84) Designated Contracting States:
CH DE FR GB IT LI NL

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(54) Humoral hypercalcemic factor antagonists.

(57) The present invention relates to the use of peptide analogues as inhibitors of their respective naturally occurring peptides. The structure of the peptide analogues is exemplified by an internal region of the N-terminus of humoral hypercalcemic factor hHCF, and truncations thereof: hHCF(14-34)NH_2, hHCF(13-34)NH_2, hHCF(12-34)NH_2, hHCF(11-34)NH_2, hHCF(10-34)NH_2, hHCF(9-34)NH_2, hHCF(8-34)NH_2, hHCF(7-34)NH_2, hHCF(6-34)NH_2, hHCF(5-34)NH_2, hHCF(4-34)NH_2, hHCF(3-34)NH_2, and these peptides containing various amino acid substitutions.

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HUMORAL HYPERCALCEMIC FACTOR ANTAGONISTS

BACKGROUND OF THE INVENTION

This invention relates to the use of peptide analogues useful in inhibiting the naturally occurring peptide in vivo and in vitro. These peptide analogues when administered to a vertebrate, such as mammals, block the activity of the peptide or other analogous molecules. These peptide analogues are also useful in vitro in combination with a bioassay for the naturally occurring peptide. The peptide analogues are useful in treating various diseases caused by excess of the naturally occurring peptide and in treating peptide dependent tumors. One example of this invention relates to the use and synthesis of humoral hypercalcemic factor (HCF) analogues useful for inhibiting the action of HCF both in vivo and in vitro.

Recently, several investigators isolated and obtained partial amino acid sequences of peptide derived from several different human tumors (lung squamous carcinoma, renal cell carcinoma, and breast carcinoma). J.M. Moseley et al., Proc. Natl. Acad. Sci. U.S.A. 84, 5048 (1987); G.J. Strewler et al., J. Clin. Invest., 80, 1803 (1987); A.F. Stewart et al., Biochem. Biophys. Res. Commun. 146, 672 (1987); M. Mangin et. al., Proc. Natl. Acad. Sci. U.S.A., 85, 597 (1988). One group published the putative full-length peptide structure (141 amino acids) based on the complementary DNA (cDNA) nucleotide sequence. L.J. Suva et al., Science 237, 893 (1987).

This new factor has been named human "humoral hypercalcemic factor" (hHCF) and is considered to be related in biological effects to parathyroid hormone (PTH). HCF shows considerable homology to the biologically critical NH_2-terminal region of PTH. However, there are significant differences in the peptide sequences between PTH and HCF, and this new factor appears to be the product of a different gene.

Previously, it had been proposed that tumors could secrete PTH ectopically and cause hypercalcemia of malignancy. However, messenger RNA for PTH was not found in such tumors. Several studies demonstrated that a PTH-like factor, physicochemically and immunologically distinct from PTH, is secreted by tumor cells. S.B. Rodan et al. J. Clin. Invest. 72, 1511 (1983); A.F. Stewart et al., Proc. Natl. Acad. Sci. U.S.A., 80, 1454 (1983); G.J. Strewler et al., J. Clin. Invest. 71, 769 (1983). It was also known that this PTH-like factor stimulates adenylate cyclase in PTH target cells, and that this activity can be inhibited by PTH antagonists. Thus, it is presently considered that HCF is a factor that is responsible for hypercalcemia of malignancy by its secretion from the tumor and its altering effect on calcium metabolism.

It is, therefore, an object of the present invention to provide antagonists of HCF. If a peptide analogue of HCF could be constructed which would bind with the cell surface receptor of HCF, then the peptide analogue could be used to block the effect of the naturally occurring peptide. Thus, it is also an object of the present invention to provide peptide analogues useful for the treatment of hypercalcemia of malignancy.

Another object of the present invention is to provide novel HCF analogues. Other objects of the present invention are to provide methods of inhibiting the action of HCF through the administration of novel HCF analogues. Still another object of the invention is to provide HCF analogues wherein amino acid modifications result in binding to the cell surface receptors without activating a second messenger molecule. The above and other objects are accomplished by the present invention in the manner more fully described below.

SUMMARY OF THE INVENTION

The present invention provides, in a first part, peptides which comprise hHCF(14-34)NH_2, hHCF(13-34)-NH_2, hHCF(12-34)NH_2, hHCF(11-34)NH_2, hHCF(10-34)NH_2, hHCF(9-34)NH_2, hHCF(8-34)NH_2.

Additionally, the first part of present invention provides the above peptides wherein (a) Ala^{34} is substituted by Tyr^{34}, and/or (b) Phe^{23} is substituted by a hydrophobic amino acid selected from the group consisting of the D- or L-stereoisomers of Leu, Nle, Val, Tyr, Trp, beta-napthylalanine and alpha-napthylalanine, and/or (c) any or each of Asp^{10}, Lys^{11} or Ile^{14} is substituted by any N-alkyl containing, or D- or L-stereoisomers of any amino acid, in particular Asn, Leu or His.

The peptides hHCF(12-34)NH_2, hHCF(11-34)NH_2, hHCF(10-34)NH_2, hHCF(9-34)NH_2, hHCF(8-34)NH_2 wherein Gly^{12} is substituted by an amino acid selected from the group consisting of the D- or L-stereoisomers of Trp, Pro, Ala, Aib, naphtyl Ala, alpha-MeTrp and NMe Gly is also an aspect of the first part of present invention. These first parts peptides wherein (a) Ala^{34} is substituted by Tyr^{34}, and/or (b) Phe^{23} is substituted by a hydrophobic amino acid selected from the group consisting of the D- or L-stereoisomers of Leu, Nle, Val, Tyr, Trp, beta-napthylalanine, alpha-napthylalanine, and/or (c) any or each of Asp^{10}, Lys^{11} or

Ile^{14} is substituted by any N-alkyl containe, or D- or L- stereoisomers of any amino acid, in particular Asn, Leu or His, are also included.

Another feature of the first part of present invention is the peptides which comprises $[Nle^{8,18}]$ hHCF (8-34) NH_2 wherein (a) Ala^{34} is substituted by Tyr^{34}, and/or (b) Phe^{23} is substituted by an amino acid selected from the group consisting of the D- or L-stereoisomers of Leu, Nle, Val, Tyr, beta-napthylalanine and alpha-napthylalanine, and/or (c) Gly^{12} is substituted by an amino acid selected from the group consisting of the D- or L- stereoisomers of Trp, Pro, Ala, Aib, naphtyl ala, alpha-MeTrp and NMe Gly, and/or (d) any or each of Asp^{10}, Lys^{11} or Ile^{14} is substituted by any N-alkyl containing, or D- or L-stereoisomers of, any amino acid, in particular Asn, Leu or His. A preferred peptide is hHCF(14-34)NH_2, and the peptide containing the substitutions indicated above where permissible.

In a second part, the present invention provides peptides which comprise hHCF(3-34)NH_2, $[Tyr^{34}]$hHCF-(3-34)NH_2, $[Nle^{8,18}$, $Tyr^{34}]$hHCF(3-34)NH_2, $[Nle^{8,18}]$hHCF(3-34)NH_2; hHCF(4-34)NH_2; $[Tyr^{34}]$hHCF(4-34)-NH_2, $[Nle^{8,18}$, $Tyr^{34}]$hHCF(4-34)NH_2, $[Nle^{8,18}]$hHCF(4-34)NH_2; hHCF(5-34)NH_2, $[Tyr^{34}]$hHCF(5-34)NH_2, $[Nle^{8,18}$, $Tyr^{34}]$hHCF(5-34)NH_2, $[Nle^{8,18}]$hHCF(5-34)NH_2; hHCF(6-34)NH_2, $[Tyr^{34}]$hHCF(6-34)NH_2, $[Nle^{8,18}$, $Tyr^{34}]$hHCF(6-34)NH_2, $[Nle^{8,18}]$hHCF(6-34)NH_2; hHCF(7-34)NH_2, $[Tyr^{34}]$hHCF(7-34)NH_2, $[Nle^{8,18}$, $Tyr^{34}]$-hHCF(7-34)NH_2, $[Nle^{8,18}]$hHCF(7-34)NH_2.

Additionally, the second part of the present invention provides the above peptides wherein (a) Gly^{12} is substituted by an amino acid selected from the group consisting of the L- or D-stereoisomers of Trp, Pro, Ala, Aib, naphthyl ala, alpha-MeTrp any NMe Gly, and/or (b) any or each of Asp^{10}, Lys^{11} or Ile^{14} is substituted by any N-alkyl containing, or D- or L- stereoisomer of any amino acid, in particular Asn, Leu or His.

Preferred peptides of the second part are $[D\text{-}Trp^{12}]$hHCF(7-34)NH_2, $[D\text{-}Trp^{12}$, $Tyr^{34}]$hHCF(7-34)NH_2, $[D\text{-}Trp^{12}$,$Nle^{8,18}$, $Tyr^{34}]$hHCF(7-34)NH_2, $[D\text{-}Trp^{12}$, $Nle^{8,18}]$hHCF(7-34)NH_2 $[Asn^{10}$, $Leu^{11}]$hHCF(7-34)NH_2, $[Asn^{10}$, Leu^{11}, $Nle^{8,18}]$hHCF(7-34)NH_2, $[Nle^{8,18}]$hHCF (7-34)NH_2, $[Asn^{10}$, Leu^{11}, $Nle^{8,18}$, $Tyr^{34}]$hHCF(7-34)-NH_2, $[Asn^{10}$, Leu^{11}, $Tyr^{34}]$hHCF(7-34)NH_2, $[Nle^{8,18}$, $Try^{34}]$hHCF(7-34)NH_2.

Also preferred are hHCF(7-34)NH_2, $[Asn^{10}$, $D\text{-}Trp^{12}]$hHCF(7-34)NH_2, $[Leu^{11}$, $D\text{-}Trp^{12}]$hHCF(7-34)NH_2,-$[Ser^{10}$, Leu^{11}, $D\text{-}Trp^{12}]$hHCF(7-34)NH_2, $[Ser^{10}$, Pro^{11}, $D\text{-}Trp^{12}]$hHCF(7-34)NH_2, $[Ser^{10}$, Sar^{11}, $D\text{-}Trp^{12}]$hHCF(7-34)NH_2, $[Leu^{11}$, $D\text{-}Trp^{12}$, $Tyr^{34}]$hHCF(7-34)NH_2.

Use of the terms "and/or" in the above description of the invention means that the substitutions described can be made singly or in any and all combinations described. For example, each of hHCF(14-34)NH_2,$[Tyr^{34}]$hHCF(14-34)NH_2, $[Leu^{23}]$ hHCF(7-34)NH_2, $[Leu^{23}$, $Tyr^{34}]$hHCF(14-34)NH_2, $[Leu^{11}]$hHCF(7-34)-NH_2, $[Leu^{11,23}]$hHCF(7-34) NH_2, $[Leu^{11,23}$,$Tyr^{34}]$hHCF(7-34)NH_2, as well as the other described combinations, are included within the present invention.

Any of the above-mentioned peptides can be used in a method of acting upon a HCF receptor which comprises administering an effective amount of such peptide to a mammal. Additionally, an in vitro bioassay of HCF, wherein a measured amount of such peptides inhibits binding of HCF to a HCF receptor in vitro is an aspect of the present invention. A pharmaceutical composition which comprises an effective amount of any such a peptide and a pharmaceutically acceptable carrier is another feature of this invention.

The present invention provides a method of inhibiting the action of HCF comprising the administration of therapeutically effective amount of HCF analogues described above. The present invention also provides a method of treating osteoporosis or hypercalcemia comprising the administration of a therapeutically effective amount of a HCF analogue described above. A method of treating hyperparathyroidism comprising the administration of a therapeutically effective amount of the HCF analogues of this invention is also provided. A method of treating hyperparathyroidism expressed as a hypercalcemic crisis, renal failure or hypertension is also provided. A method of treating the disease state produced by a tumor or other cell overproducing a peptide hormone-like molecule and method of treating immune diseases wherein the disease state comprises inflammation, an allergic response, or hyperactive lymphocytes is also provided by the novel peptide analogues of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

Various other objects, features and attendent advantages of the present invention will be more fully appreciated as the same becomes better understood from the following detailed description.

Extensive structure and activity studies have now led to the design of peptide analogues which have high binding affinity for their respective cell surface receptors while not stimulating the production of second messenger molecules.

HCF analogues with two to thirteen amino acids removed from the N-terminus produces an inhibitor

which still binds with high affinity to the peptide hormone receptor without causing a change in cyclic AMP concentration.

The following is the 34-amino acid sequence of human humoral hypercalcemia factor (hHCF):
Ala-Val-Ser-Glu-His(5)-Gln-Leu-Leu-His-Asp(10)-Lys-Gly-Lys-Ser-Ile(15)-Gln-Asp-Leu-Arg-Arg-(20)-Arg-Phe-Phe-Leu-His(25)-His-Leu-Ile-Ala-Gln(30)-Ile-His-Thr-Ala. Standard abbreviations well recognized in the peptide chemistry art are utilized herein. Aib represents an aminoisobutyrl substitutent.

Fragments of peptide, containing the region specific for binding to the cell surface receptor can be used as inhibitors or blocking agents. For HCF, it is considered that the N-terminal 34 amino acids are sufficient to define binding specificity to the cell surface receptor.

The presence of D-amino acids in peptide in place of L-amino acids sometimes results in a peptide resistant to catabolism. However, not all such substitutions result in an active peptide. Thus, such substitutions which result in active peptide are considered to be within the scope of the present invention. The utilization of D-amino acids in peptide hormone synthesis is described in the following publications herein incorporated by reference: Coltrera, et al., Biochemistry, 19:4380-4385, 1980; Rosenblatt et al., Biochemistry, 20:7246-7250, 1981. Additionally, substitutions of amino acids which are equivalent to the amino acids disclosed herein is considered to be within the scope of the present invention.

The balance of the description will be divided into two sections. Section I will describe the preparation and structure of inhibitors of peptide hormones, Section II will discuss the use of the peptide hormone inhibitors.

I. Preparation and Structure of Peptide Hormone Inhibitors

The technique of solid-phase peptide synthesis, developed by Merrifield ("Solid-Phase Peptide Synthesis", Advances in Enzymology, 32:221-296, (1969); G. Barany and R.B. Merrifield "Solid-Phase Peptide Synthesis" in The Peptides, volume 2, editors: E. Gross & J. Meienhofer (1980)) has been successfully employed in the synthesis of peptides including HCF. This method is based on the strategy of having the carboxyl terminus of the peptide linked covalently to a solid support. The desired peptide sequence is prepared by stepwise coupling of single amino acids to a peptide chain growing from the carboxyl toward the amino terminus. Coupling is typically achieved by activation of the carboxyl group of the amino acid being attached to the resin, which may have other potentially reactive groups blocked. Following addition of an amino acid to the growing polypeptide chain, and prior to further chain elongation, the alpha-amino (Boc) protecting group is typically removed. Because each amino acid is coupled by nearly the same series of reactions, the need for elaborate strategies in the synthesis is minimized. Solublility is not a major issue during synthesis, because the peptide is linked to a solid support. This method is rapid and it can be utilized by a single worker. It is very convenient for the synthesis of multiple analogues with amino-terminal substitutions, because a single synthesis can be branched in multiple directions near the amino terminus, thereby creating many analogues varying only in the amino terminal region.

II. Use of Peptide Inhibitors

The method of inhibiting the action of HCF peptide comprises the administration of a therapeutically effective amount of any HCF peptide analogue. These peptide analogues retain specificity for the cell surface receptor without stimulating a physiological response. This method of use applies to the entire peptide or its analogue, or to a fragment of the peptide or analogue containing the receptor binding site.

The use of peptide analogues is exemplified by HCF analogues. The HCF is of human origin but HCF of bovine, rat or any mammalian source may prove to be equivalent to the human HCF. The analogue may contain all the amino acids indicated, or additionally truncations or elongations. Individual amino acids can be substituted to improve biological or chemical stability.

The peptide analogues of this invention can be used in vitro to measure the concentration of naturally occurring peptide. This bioassay procedure is illustrated by a bioassay for HCF. The unknown concentration of HCF in a solution can be determined by measuring the amount of HCF analogue required to inhibit its binding to the HCF cell surface receptor. The concentration of HCF analogue required to block the action of HCF is a direct indicator of the HCF concentration.

HCF analogues can be used to diagnose the etiology of or to treat osteoporosis or hypercalcemia through the administration of a therapeutically effective amount of the HCF analogues of this invention. Similarly, hyperparathyroidism and other aspects of hyperparathyroidism, such as a hypercalcemic crisis,

renal failure or hypertension can be treated through the administration of the HCF analogues of this invention.

Tumors and other aberrant cell growth often produce hormone-like substances causing a disease state. The use of peptide analogues to block stimulation caused by such hormone like substances can result in the alleviation of the disease state. An example of this is the humoral hypercalcemic factor of malignancy. Therefore, the HCF peptide analogues of the present invention can be administered to treat diseases caused by aberrant production of hormone-like substances.

The peptide analogues of this invention exhibit both oral and parenteral activity and can be formulated in dosage forms for oral, parenteral, intra-nasal, or topical administration. Solid dosage forms for oral administration include capsules, tablets, pills, powders and granules. In such solid dosage forms, the active compound is admixed with at least one inert diluent such as sucrose, lactose or starch. Such dosage forms can also comprise, as is normal practice, additional substances other than inert diluent. In the case of capsules, tablets, and pills, the dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with an enteric coating.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsion, solutions, suspensions, syrups and elixers containing inert diluents commonly used in the pharmaceutical art. Besides inert diluents, such compositions can also include adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening. Preparations according to this invention for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions or emulsions. Examples of non-aqueous solvents or vehicles are propylene glycol, polyethylene glycol, vegetable oils such as olive oil and injectable organic esters such as ethyloleate.

The dosage of active ingredient in the compositions of this invention may be varied; however it is necessary the amount of the active ingredient shall be such that a suitable dosage form is obtained. The selected dosage form depends upon the desired therapeutic effect, on the route of the administration, and on the duration of the treatment.

Obviously, numerous modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described herein.

EXAMPLE 1

Synthesis and Purification of Peptide Analogues of HCF

Analogues of HCF, where prepared by a modification of the solid-phase method of Merrifield. Syntheses were performed using an Applied Biosystems 430A Synthesizer. 4-Methyl-benzhydrylamine hydrochloride resin (polystyrene-1% by divinylbenzene, USB) was employed as the solid support in order to effect the carboxyamide (CONH2) COOH-terminal modification.

The tertiary butyloxycarbonyl (Boc) group was used to protect the alpha -amino group of each amino acid during coupling. Side-function protection was afforded as follows: (a) the hydroxyl group of serine was protected as the O-benzyl ether (Bzl); (b) the hydroxyl group of tryosine as the 0-2,6-dichlorobenzyl ether (DCB) or p-bromobenzyloxycarbonyl ester (Brz); (c) the carboxyl group of glutamic and aspartic acid as the benzyl (BZ) or cyclohexyl ester (Chx); (d) the imidazole nitrogen of histidine by the benzyloxymethyl (BOM) and the guanidine function of arginine was protected by the p-toluene-sulfonyl (TOS) group, and the indole imine by formyl groups (For); and (e) the Lysine epsilon amino group by 2-chloro-benzyloxycarboxyl (ClZ). All amino acids were obtained from Applied Biosystems, Inc., Peninsula Laboratories, or Bachem Chemicals.

The peptide-resin synthesis were carried out using Applied Biosystems, Inc. specified protocols. Double couplings were carried out for the incorporation of each amino acid. After the final coupling of each of the arginines (residues 18-21) the remaining free amino groups were acetylated to prevent generation of deletion peptides. Deprotection times with trifluoroacetic acid (TFA) were extended 6 minutes over manufacturer protocols.

The peptide was cleaved from the copolymer resin with simultaneous removal of the side-chain protecting groups similar to the 2 step HF cleavage procedure described by Tam, J.A.C.S. 105: 6442-6455 (1983). In the first HF step the following ratios of reagents were used: 5% p-cresol, 5% p-thiocresol, 65%

dimethyl sulfide and 25% HF. 10 ml of mixture per gram of peptide-resin was used for 2 hours at 0 °C. In the second HF step the following ratio of reagents were used: 5% p-cresol, 5% p-thiocresol and 90% HF. The cleavage was carried out for 75 min. at 0 °C. After removal of HF the peptide-resin mixture was washed with anhydrous ether to remove scavenger. The peptide was then extracted with 50% acetic acid and water. The washes were combined and chromatographed using Sephadex G-50F, eluting with 50% HOAc.

After lyophilization, the partially purified peptide was chromatographed by reverse phase HPLC (Vydac C_4 bonded silica, 15 u particle size, 300A pore size, using aqueous acetonitrile gradient containing 0.1%TFA).

EXAMPLE 2

HCF Binding Assay Results

HCF analogues were analysed in a new receptor assay (Goldman et al., Emdrpcrinol (1988) 123: 1468-1475) which modified the assay reported in Rosenblatt et al., Endocrin. 107: 545-550 (1980). The binding assay used $[Nle^{8,18}(^{125}I)\text{-}Tyr^{34}]$bPTH (1-34)$NH_2$ which was purified by HPLC (Novapak C_{18}, 32-35% CH_3CN in 0.1% TFA) and was stored as aliquots in 25 mM TrisHCl/1%BSA at -70 °C. Bovine renal cortical plasma membranes were incubated with radioligand (25,000 cpm) in the absence or presence of HCF analogs in a Tris-containing buffer (250 ul) for 30 min. at 21 °C. Once equilibrium was reached, bound and free radioligand were separated by centrifugation. High specific binding (85%) to bovine renal cortical membranes was obtained consistently.

Structure	Binding K_i(nM)
hHCF(14-34)NH_2	1400 ± 83
[Asn^{10},Leu^{11}]hHCF(7-34)NH_2	104 ± 27
hHCF(7-34)NH_2	257 ± 37
[D-Trp^{12}]hHCF(7-34)NH_2	50 ± 14
[Asn^{10},D-Trp^{12}]hHCF(7-34)NH_2	39 ± 15
[Leu^{11},D-Trp^{12}]hHCF(7-34)NH_2	4 ± 2
[Asn^{10},Leu^{11},D-Trp^{12}]hHCF(7-34)NH_2	7 ± 2
[Ser^{10},Leu^{11},D-Trp^{12}]hHCF(7-34)NH_2	39 ± 15
[Ser^{10},Pro^{11},D-Trp^{12}]hHCF(7-34)NH_2	113 ± 38
[Ser^{10},SAR^{11},D-Trp^{12}]hHCF(7-34)NH_2	14 ± 5
[D-Trp^{12}]hHCF(10-34)NH_2	403 ± 251

EXAMPLE 3

HCF analogues were analyzed in a bovine renal membrane adenylate cyclase assay as described in Horiuchi et al., Science 238, 1566 (1987); Goldman et al., Endocrin (1988) 123(5): 1468-1475. 3nM $[Nle^{8,18}\text{-}Tyr^{34}]$bPTH(1-34) NH_2 was used to stimulate adenylate cyclase.

Structure	Binding K_i(nM)
hHCF(14-34)NH_2	6,600 ± 1300
[D-Trp[12]]hHCF(10-34)NH_2	2,365 ± 650
hHCF(7-34)NH_2	609 ± 133
[D-Trp[12]]hHCF(7-34)NH_2	390 ± 183
[Asn[10],D-Trp[12]]hHCF(7-34)NH_2	244 ± 87
[Leu[11],D-Trp[12]]hHCF(7-34)NH_2	33
[Asn[10],Leu[11],D-Trp[12]]hHCF(7-34)NH_2	73 ± 16
[Ser[10],Leu[11],D-Trp[12]]hHCF(7-34)NH_2	244 ± 87
[Ser[10],Pro[11],D-Trp[12]]hHCF(7-34)NH_2	1093 ± 430

EXAMPLE 4

HCF analogues were analyzed in a rat osteosarcoma cell line, ROS 17/2.8, for the ability to inhibit cAMP stimulation by 1nM [$Nle^{8,18}$,Tyr^{34}]bPTH(1-34) NH_2 by the method described by McKee, R. et al, Endocrinol. (1988) 122(6): 3008-10.

Structure	Binding K_i(nM)
hHCF(14-34)NH_2	2040 ± 160
hHCF(7-34)NH_2	90 ± 220
[Asn[10],Leu[11]]hHCF(7-34)NH_2	37 ± 2
[D-Trp[12]]hHCF(7-34)NH_2	132 ± 12
[Asn[10],D-Trp[12]]hHCF(7-34)NH_2	74 ± 9
[Leu[11],D-Trp[12]]hHCF(7-34)NH_2	6.6 ± 0.9
[Asn[10],Leu[11],D-Trp[12]]hHCF(7-34)NH_2	8.9 ± 1.5
[Ser[10],Leu[11],D-Trp[12]]hHCF(7-34)NH_2	8.6 ± 2.7
[Ser[10],Pro[11],D-Trp[12]]hHCF(7-34)NH_2	51 ± 5

Example 5

HCF analogues were assayed for their ability to inhibit PTH-stimulated erdenylate cyclose in human osteosarcoma cells (B10) in a similar manner to that described in Example 4.

Structure	Binding K_i(nM)
hHCF(7-34)NH_2	77 ± 11
[Asn[10],Leu[11]]hHCF(7-34)NH_2	9
[D-Trp[12]]hHCF(7-34)NH_2 17 ± 6	
[Asn[10],D-Trp[12]]hHCF(7-34)NH_2	3.5
[Leu[11],D-Trp[12]]hHCF(7-34)NH_2	2.2 ± 0.4
[Leu[11],D-Trp[12]Tyr[34]]) 5.9 ± 0.9 hHCF(7-34)NH_2	
[Asn[10],Leu[11],D-Trp[12]] 3.5 ± 0.2 hHCF(7-34)NH_2	
[Ser[10],Leu[11],D-Trp[12]] 4.0 ± 0.7 hHCF(7-34)NH_2	

Example 6

HCF analogues were assayed for their ability to inhibit 1^{125}-labeles PTH binding to plasma membranes derived from human osteosaroma cell (B10) in a manner similar to that described in Example 2.

Structure	Binding K_i(nM)
hHCF(7-34)NH_2	60
[Leu^{11}, [D-Trp^{12}]hHCF(7-34)NH_2	10
[Asn^{10},Leu^{11},D-Trp^{12}]hHCF(7-34)NH_2	

Claims

1. A peptide which comprises hHCF(14-34)NH_2, hHCF(13-34), hHCF(12-34)NH_2, hHCF(11-34)NH_2, hHCF(10-34)NH_2, hHCF(9-34)NH_2, hHCF(8-34)NH_2.

2. A peptide of Claim 1 wherein (a) Ala^{34} is substituted by Tyr^{34}, and/or (b) Phe^{23} is substituted by an amino acid selected from the group consisting of the D- or L-stereoisomers of Leu, Nle, Val, Tyr, Trp, beta-napthylalanine and alpha-napthylalanine and/or (c) any or each of Asp^{10}, Lys^{11} or Ile^{14} is substituted by any N-alkyl containing, or D- or L-stereoisomers of any amino acid, in particular Asn, Leu and His.

3. A peptide which comprises hHCF(12-34)NH_2, hHCF(11-34)NH_2, hHCF(10-34)NH_2, hHCF(9-34)NH, hHCF(8-34)NH_2 wherein Gly^{12} is substituted by an amino acid selected from the group consisting of the D- or L-stereoisomers of Trp, Pro, Ala, Aib, naphtyl Ala, alpha-MeTrp and NMeGly.

4. A peptide of Claim 3 wherein (a) Ala^{34} is substituted by Tyr^{34}, and/or (b) Phe^{23} is substituted by an amino acid selected from the group consisting of the D- or L-stereoisomers of Phe, Leu, Nle, Val, Tyr, beta-napthylalanine and alpha-napthylalanine, and/or (c) any or each of Asp^{10}, Lys^{11} or Ile^{14} is substituted by any N-alkyl containing, or D- or L-stereoisomers of, any amino acid, in particular Asn, Leu or His.

5. A peptide which comprises [$Nle^{8,18}$] hHCF(8-34) NH_2 wherein (a) Ala^{34} is substituted by Tyr^{34}, and/or (b) Phe^{23} is substituted by an amino acid selected from the group consisting of the D- or L-stereoisomers of Leu, Nle, Val, Tyr, Trp, beta-napthylalanine and alpha-napthylalanine, and/or (c) Asp^{10} or Lys^{11} is substituted by any N-alkyl containing, or D- or L-stereoisomers of any amino acid, in particular Asn, Leu or His, and/or (d) Gly^{12} is substituted by an amino acid selected from the group consisting of D- or L-stereoisomers of Trp, Pro, Ala, Aib, and NMeGly.

6. A peptide of Claim 1 which is hHCF(14-34)NH_2.

7. A peptide of Claim 3 which is [D-Trp^{12}]hHCF(10-34)NH_2.

8. A peptide which comprises hHCF(3-34)NH_2, [Tyr^{34}]hHCF(3-34)NH_2, [$Nle^{8,18}$, Tyr^{34}]hHCF(3-34)NH_2, [$Nle^{8,18}$]hHCF(3-34)NH_2; hHCF(4-34)NH_2; [Tyr^{34}]hHCF(4-34)NH_2, [$Nle^{8,18}$, Tyr^{34}]hHCF(4-34)NH_2, [$Nle^{8,18}$]-hHCF(4-34)NH_2; hHCF(5-34)NH_2, [Tyr^{34}]hHCF(5-34)NH_2, [$Nle^{8,18}$, Tyr^{34}]hHCF(5-34)NH_2, [$Nle^{8,18}$]hHCF(5-34)NH_2; hHCF(6-34)NH_2, [Tyr^{34}]hHCF(6-34)NH_2, [$Nle^{8,18}$, Tyr^{34}]hHCF(6-34)NH_2, [$Nle^{8,18}$]hHCF(6-34)NH_2; hHCF(7-34)NH_2, [Tyr^{34}]hHCF(7-34)NH_2, [$Nle^{8,18}$, Tyr^{34}]hHCF(7-34)NH_2, [$Nle^{8,18}$]hHCF(7-34)NH_2.

9. A peptide of Claim 8 where (a) Gly^{12} is substituted by an amino acid selected from the group consisting of the L- or D- stereoisomers of Trp, Pro, Ala, Aib, naphtyl Ala, alpha-MeTrp and NMeGly, and/or (b) any one or more of Asp^{10}, Lys^{11} or Ile^{14} is substituted by any N-alkyl containing, or D- L- stereoisomers of, any amino acid, in particular Asn, Leu or His.

10. A peptide of Claim 8 or 9 which is [D-Trp^{12}]hHCF(7-34)NH_2, [D-Trp^{12}, Tyr^{34}]hHCF(7-34)NH_2, [D-Trp^{12}, $Nle^{8,18}$, Tyr^{34}]hHCF(7-34)NH_2, [D-Trp^{12}, $Nle^{8,18}$]hHCF(7-34)NH_2, [Asn^{10}, Leu^{11}]hHCF(7-34)NH_2,-[Asn^{13}, D-Trp^{12}]hHCF(7-34)NH_2,[Ser^{10}, Leu^{11}, D-Trp^{12}]hHCF(7-34)NH_2, [Ser^{10}, Pro^{11}, D-Trp^{12}]hHCF(7-34)-NH_2, [Ser^{10}, Sar^{11}, D-Trp^{12}]hHCF(7-34)NH_2, [Leu^{11}, D-Trp^{12}, Tyr^{34}]hHCF(7-34)NH_2.

11. The use of a peptide as claimed in any one of claims 1 to 10 for the preparation of a medicament useful for acting upon a HCF receptor in mammals.

12. A pharmaceutical composition which comprises an effective amount of a peptide of any of Claims 1 to 10 and a pharmaceutically acceptable carrier.